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The Raccoon (*Procyon lotor*) on St. Catherines Island, Georgia. 3. Presence of Carotenoids in Blood

MARY ELLEN HARDIN[1]

ABSTRACT

Sixty-three blood samples were obtained by cardiac puncture from 53 raccoons from St. Catherines Island, Georgia, from January 20 to June 4, 1975 and from January 6 to 16, 1976. Blood was mixed with an equal volume of 10 percent aqueous trichloro-acetic acid to extract carotenoid pigments. The supernate was scanned with a Beckman double beam-grating spectrophotometer between wavelengths of 700 and 200 nm. Absorption maxima for carotenoids were adjusted and compared with the following independent variables: age, sex, and weight of the raccoons, season of the year during which blood was taken, and location of capture.

All blood samples obtained from raccoons from St. Catherines Island contained carotenoids. None of the independent variables accounted for a significant amount of carotenoid variability. Further study of trace elements and environmental factors that may influence pelage color is warranted.

INTRODUCTION

Raccoons of the subspecies *Procyon lotor litoreus* are abundant on St. Catherines Island, Liberty County, Georgia. These animals are distributed throughout the Sea Islands in every habitat type (Johnson et al., 1974). Raccoons occurred in all habitats. The vegetation of the island was mapped by Somes and Ashbaugh (1973) and represents six general physiognomic types: grasslands (including tidal marshes, meadows, and upland grasslands), savanna, forest, scrub, herblands, and aquatics.

Raccoon coloration ranges from the normal brown to silver and to a reddish orange; the redder animals are said to be more common in marshes on St. Catherines Island and elsewhere. Xanthochromistic pigmentation is not unique to raccoons of St. Catherines Island. Dozier (1948) reported that *P. l. maritimus* of salt marshes were pale in color and blended with marsh vegetation. The lighter color was a result of a prominent, pale subapical band of guard hairs which was exposed and projected farther beyond the underfur than it did in typical specimens of *P. l. lotor* (Dozier, Hardy and Markley, 1948). Louisiana "salt water" raccoons had reddish to red-brown hair. Bachrach (1953) speculated that this coloration was due "to the food they eat." Ivey (1948) reported northeastern Florida raccoons (*P. l. elucus*) that were light to reddish colored possibly because

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of exposure to sun in treeless marshes. Thus, food and sunlight have been thought to increase xanthochromatism in raccoons.

Variations in coat color may result from at least two sets of differences in diet. Trace elements affect hair structure (copper, molybdenum, zinc) and may influence pigmentation as well (Underwood, 1956). Another explanation is that color is influenced by plant pigments ingested in food. Carotenoids are among the most widely distributed of all pigments and are a group of yellow, orange, red, or violet compounds found in both animals and plants. Animals cannot synthesize carotenoids and, therefore, must depend on their diet as a source of these compounds (Fox, 1953; Bagnara and Hadley, 1973). Ingested carotenoids impart color to numerous invertebrates and to some members of the Osteichthyes, Amphibia, Reptilia, and Aves (Fox, 1953, 1962; Needham, 1974). Little work has been done on the influence of carotenoid pigments on color of mammalian pelage. These pigments have been isolated from ovaries, eggs, liver, blood, and integument of mammals and remain unchanged from plant to animal tissue component.

Needham (1974) reported that members of the order Carnivora do not absorb carotenoids from the gut and that carotenoids are rarely found in tissues of these animals. Fox (1953) reported that "carnivorous and certain other mammals" store little or none of these pigments in fat or other tissues and contain none in their blood (Palmer, 1922; Zechmeister, 1937; Goodwin, Dewar and Gregory, 1946). However, carotenoids are found in blood plasma of some species. Here, the pigments, absorbed from the food, are in transit to places of temporary storage where they form a carotene-protein complex which is water soluble and fat insoluble. In these species, carotenes have been recovered from liver, lungs, spleen (Drummond, Gilding and MacWalter, 1934) and adrenals (Fox, 1953). See Lotze and Fleischman (1978) for other blood values.

The relative amounts of carotenoids in raccoon blood and the usefulness of certain variables in predicting levels of carotenoids was tested. The variables tested were age, sex, and weight of the animals, season of the year during which blood was taken, and location of capture. Because the reddish color morph seemed to be concentrated in marsh areas, least distance to a marsh from each point of capture was correlated with carotenoid content to determine if reddish animals had higher carotenoid levels.

ACKNOWLEDGMENTS

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STUDY AREA

St. Catherines Island forms the ocean edge of Liberty County, Georgia. The island of about 5800 hectares (14,000 acres) is near the midpoint of a sea island chain extending along the coast of northern Florida, Georgia, and southern South Carolina. St. Catherines Island is 8.2 km. (5.1 mi.) from the mainland, approximately 64.5 km. (40 mi.) southeast of Savannah, Georgia, and is 16.5 km. (10.3 mi.) long and 6.3 km. (3.9 mi., maximum) in width. (See also Hudson, 1978.)

The origin of the Sea Islands has been reviewed by Thornbury (1965), Johnson et al. (1974) and Somes and Ashbaugh (1973). St. Catherines Island was formed during the Pleistocene while vast quantities of sea water were taken up by glaciers. Dunes formed along continental beaches and remained stable while

the area flooded with sea water as the glaciers melted. These dunes were shaped by erosion and are characterized by irregular outlines, nearly level topography, and sandy sediments upon which a mature soil profile has developed. Tidal marshes developed on the landward and seaward sides of the erosion remnants during the last 5000 years and contain substantial amounts of organic material; surface elevation is near mean sea level. Beach ridges of aeolian sands formed on the seaward edge of the tidal marsh. This topography is characterized by parallel, arcuate dune ridges to 8 m. (25 ft.) and a very immature soil profile.

MATERIALS AND METHODS

In order to obtain blood samples from free-ranging raccoons, Tomahawk live-traps (255 by 305 by 760 mm.) were positioned at marked intervals of about 0.16 km. (0.1 mi.) mostly along existing roadways (fig. 1). Traps were set daily and baited with cat food or table scraps from January 20 to June 4, 1975 and from January 6 through 16, 1976. Harman trapped from January 20 to May 20, 1975. Traps were baited in late afternoon and checked routinely before 1000 hours the next morning and sometimes in late evening between 2100 and 2400 hours. Although traps were not positioned in a completely random fashion, they were in grassland, marsh edge, meadow, savanna, forest, and scrub habitats; thus they sampled all major habitats of the island.

Location of capture was recorded and then raccoons were transported to a laboratory where ketamine hydrochloride (Ketaset) was administered (0.2 cm^3/kg. body weight). Ten cm^3 of blood was obtained by cardiac puncture from the anesthetized animal, which was aged, sexed, weighed, and (after recovery from anesthesia within two to four hours) released at the point of capture. Two animals were held captive overnight for repeated blood sampling.

Each of the 42 whole blood samples (collected from May 20 to June 4, 1975 and from January 6 to 16, 1976) was mixed with an equal volume of 10 percent aqueous trichloro-acetic acid (TCA); TCA precipitated long chain proteins, and upon centrifugation allowed pigments and amino acids to be removed in the supernate

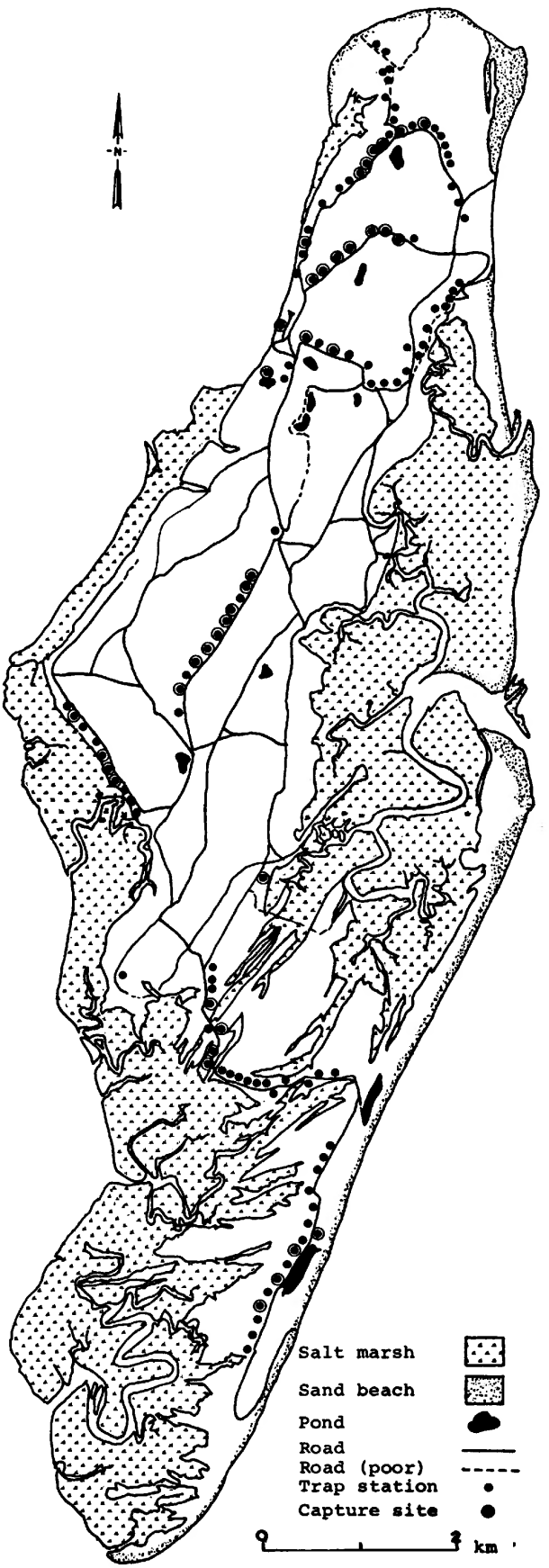

FIG. 1. Trap stations and capture locations for raccoons from St. Catherines Island, Georgia.

(Yopp, personal commun.). The supernate from each sample was refrigerated in an airtight vial in the absence of light until analyzed. Twenty-one other whole blood samples collected by Harman (from January 21 to May 20, 1975) were frozen immediately. These were mixed with an equal volume of 10 percent TCA while thawing, centrifuged, and stored as above. Prior to analysis, samples were recentrifuged and then scanned with a Beckman double beam-grating spectrophotometer (Model DB-GT) between wavelengths of 700 and 200 nm. Absorption maxima between 390 and 395 nm., within the range of peak light absorption for carotenoids (Weast, 1976), were graphed. In order to minimize any variations in the spectrophotometer or other extrinsic factors, values were adjusted by subtracting absorbance at 700 nm. from absorbance at 390 nm., thus, no units were expressed.

Animals were aged according to total length, weight, and tooth wear as reported by Johnson (1970). Some of the animals used in this study (nos. 43 to 60, 62, 63) were prepared as whole specimens and aged by Harman using the following criteria: eye lens weight (Sanderson, 1961; Johnson, 1970), cranial suture closure (Grau, Sanderson and Rogers, 1970), epiphyseal cartilage development (Sanderson, 1961), and baculum development (Sanderson, 1950). Harman has found these techniques reliable for separating raccoons of St. Catherines Island into adult (more than two years of age), subadult (one to two years of age), and juvenile (less than one year) age classes.

Winter samples were collected from January 1 to February 28. Those collected from March 1 to June 5 were spring samples. The first of March was the mean date of the last 0°C. (32°F.) day in spring for St. Catherines Island (Environmental Data Service, 1966) and was designated the first day of spring for purposes of analysis. Least distance to a marsh was determined by plotting each capture location on a topographic map (scale 1:24000) and measuring distance to the nearest marsh.

The relationships between adjusted carotenoid content and age, sex, and weight of the raccoons, season of the year during which blood was obtained, and location of capture, were tested by the Statistical Package for Social Sciences (SPSS) programs (Nie et al., 1975). Independent variables were coded: age (adult, subadult, juvenile), sex (0-male, 1-female), weight (kg.), season of the year (0-winter, 1-spring), and least distance to a marsh (km.). Carotenoid content of blood was designated the dependent variable. Coefficients were calculated to determine degree of correlation between all variables. Multiple regression analysis was used to determine the contribution of the independent variables to the variation in carotenoid content. Multiple regression analysis was used on first order interactions to determine if any two variables acted in conjunction to affect carotenoid level. The interaction terms were: season-age, season-sex, season-weight, season-distance, age-sex, age-weight, age-distance, sex-weight, sex-distance, and weight-distance. Higher order interactions were not calculated due to the small sample size (n=57).

Residuals were plotted to determine any nonlinear relationships in the data; as none was found, higher order polynomial terms were not used in the regression equation. The alpha level for all statistical tests was set at 0.05.

Attempts were made to extract carotenoids from hair of 22 St. Catherines Island raccoons. Extraction procedures were modified from Gortner (1910), Nickerson (1946), Fox (1953), and Fox and Hopkins (1965). Hair was treated with KOH and heated for 15 minutes until the hair dissolved. The heating process necessary to denature the hair proteins destroyed the carotenoids and none was recovered.

RESULTS

Of the 63 blood samples collected, those included in the statistical analyses were obtained from 14 adult males, two subadult males, three adult females, one subadult female, and nine juvenile females sampled in winter. One adult male, one subadult male, and one juvenile female were recaptured and resampled. The spring sampling consisted of nine adult males, seven subadult males, six adult females, and two subadult females. One adult male was recaptured and resampled. Six blood samples were not used in the statistical anal-

yses. Two (nos. 36, 39), taken in spring, were not included because sufficient information was not available to age the animals. Four samples, obtained in winter, were not used since the animals remained in the laboratory before blood was taken. These animals were either denied food (nos. 3, 21) or maintained on an artificial diet (nos. 22, 23) and will be discussed below.

All blood samples obtained from raccoons from St. Catherines Island contained carotenoids; adjusted levels ranged from 0.020 to 1.612 (table 2). The mean level was 0.329 (s.d.=0.257, n=57). Correlations between carotenoid content and adult, subadult, and juvenile age classes, sex, weight, season, and location of capture are shown in table 1. These results, which were statistically nonsignificant, indicated only a weak relationship between carotenoid content and the independent variables.

Multiple regression analysis yielded R^2 values for each of the independent variables (table 3). Age, sex, weight, season, and location of capture accounted for 14.8 percent of the variability in carotenoid level. This regression analysis did not reveal any of the independent variables contributing a statistically significant amount to carotenoid variability.

First order interactions alone accounted for 11.2 percent of the carotenoid variability (table 3); none of the interactions was significant. Season-juvenile and juvenile-sex interactions were not calculated because of perfect correlations (r=1.0) in these categories, as all juveniles were female and captured in winter. Residual scores were plotted; values seemed to be randomly distributed, and, therefore, no curvilinear relationships were suggested (fig. 2).

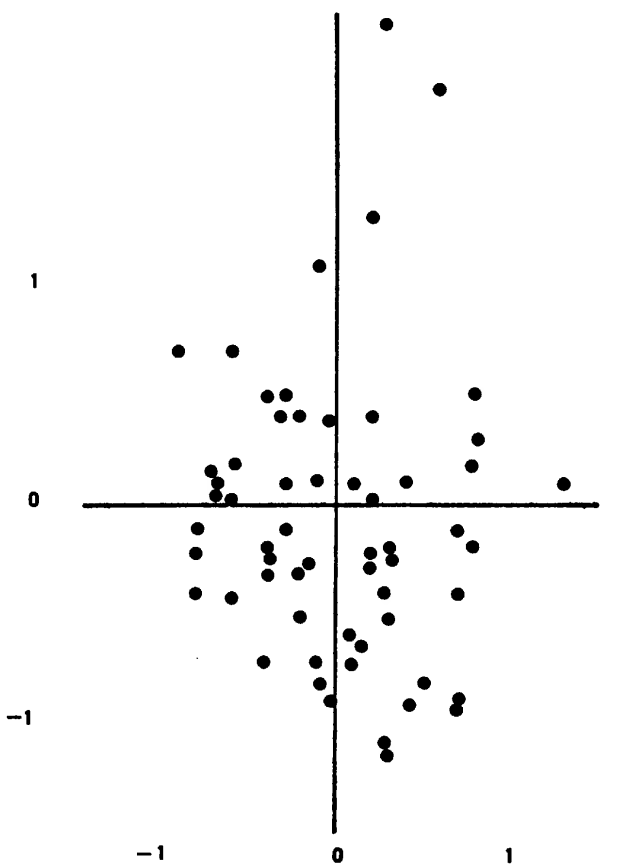

FIG. 2. Distribution of residual values of predicted carotenoid content from raccoons from St. Catherines Island, Georgia; *x* axis is predicted standardized dependent variable, *y* axis is standardized residual.

DISCUSSION

Raccoons of St. Catherines Island have carotenoids in their blood, contrary to reports that carotenoids rarely are found in tissues of members of the Carnivora (Fox, 1953; Needham, 1974). Relative amounts of carotenoids ranged from 0.02 to more than 80 times that value and were not affected by age, sex, weight of the animal, season during which blood was obtained, or location of capture.

The variables tested did not interact to affect nor account for a significant amount of carotenoid variability. Significant correlations were expected between weights of animals and their age, sex, and season of the year based on Stains (1956), Johnson (1970), and previous literature on the natural history of raccoons. Other significant results indicated more subadults were captured in spring and most of the adult raccoons captured were male. Since all juvenile animals sampled were females captured in winter, a significant relationship was found between juvenile age class and sex, and season.

Female raccoons failed to show higher carotenoid levels than males. Among the various species of vertebrates and invertebrates studied, females generally had greater carotene con-

TABLE 1

Correlation Coefficients Between Selected Data from Raccoons from St. Catherines Island, Georgia

	Season	Adult	Subadult	Juvenile	Sex	Weight	Distance	Carotene
Season	—	0.078	0.278[a]	−0.408[a]	−0.120	−0.292[a]	0.234	−0.166
Adult	—	—	−0.661[a]	−0.561[a]	−0.303[a]	0.624[a]	0.003	0.037
Subadult	—	—	—	−0.251	−0.173	−0.328[a]	0.033	−0.056
Juvenile	—	—	—	—	0.582[a]	−0.443[a]	−0.041	0.014
Sex	—	—	—	—	—	−0.668[a]	0.019	−0.006
Weight	—	—	—	—	—	—	−0.110	−0.036
Distance	—	—	—	—	—	—	—	−0.234

[a]Significant at 0.05.

TABLE 2

Selected Data from Raccoons of St. Catherines Island, Georgia

Specimen Number	Eartag Number	Season[a] Year	Age	Sex	Weight (kg.)	Distance (km.)	Carotene Content
1	GA001	W 1976	Adul	M	5.1	0.05	0.161
2	237	W 1976	Adul	M	5.1	1.40	0.245
3	237	W 1976	Adul	M	5.1	Held	0.195
4	245	W 1976	Adul	M	5.6	0.25	0.110
5	249	W 1976	Adul	F	3.3	1.10	0.310
6	261	W 1976	Adul	M	4.8	0.05	0.340
7	298	W 1976	Suba	M	3.6	0.90	0.181
8	298	W 1976	Suba	M	3.4	0.65	0.368
9	311	W 1976	Adul	M	5.7	0.40	0.102
10	504	W 1976	Adul	M	5.8	0.75	0.208
11	609	W 1976	Adul	M	5.2	0.70	1.612
12	609	W 1976	Adul	M	4.9	0.65	0.125
13	616	W 1976	Juve	F	2.8	0.80	0.352
14	616	W 1976	Juve	F	2.8	0.80	0.045
15	617	W 1976	Juve	F	2.6	0.80	0.272
16	618	W 1976	Juve	F	2.5	0,80	0.380
17	619	W 1976	Juve	F	2.0	0.05	0.412
18	620	W 1976	Juve	F	2.2	1.10	0.356
19	621	W 1976	Juve	F	3.0	0.05	0.110
20	622	W 1976	Adul	M	5.9	0.35	0.320
21	622	W 1976	Adul	M	5.6	Held	0.241
22	622	W 1976	Adul	M		Held	0.361
23	622	W 1976	Adul	M	5.3	Held	0.400
24	623	W 1976	Juve	F	2.2	0.05	0.962
25	624	W 1976	Juve	F	2.2	0.70	0.185
26	625	W 1976	Adul	M	5.0	0.05	0.378
27	628	W 1976	Adul	M	5.5	0.05	0.585
28	DH646	W 1976	Adul	M	3.4	1.10	0.482
29	DH647	W 1976	Adul	F	3.0	0.05	0.688
30	237	S 1975	Adul	M	4.2	1.00	0.238
31	237	S 1975	Adul	M	3.8	0.85	0.210
32	245	S 1975	Adul	M	3.7	0.60	0.299
33	291	S 1975	Suba	M	3.4	0.85	0.170
34	338	S 1975	Suba	F	2.1	1.00	0.167

TABLE 2 — (*Continued*)

Specimen Number	Eartag Number	Season[a] Year	Age	Sex	Weight (kg.)	Distance (km.)	Carotene Content
35	340	S 1975	Suba	M	2.0	1.65	0.632
36	540	S 1975		M		0.70	0.275
37	577	S 1975	Adul	M	3.7	1.35	0.020
38	579	S 1975	Suba	F	2.4	0.80	0.367
39	585	S 1975		M		0.05	1.032
40	586	S 1975	Adul	F	2.5	0.85	0.269
41	587	S 1975	Suba	M	3.4	0.10	0.173
42	588	S 1975	Adul	M	2.7	1.00	0.472
43	DH291	W 1975	Adul	M	4.7	0.01	0.120
44	DH292	W 1975	Suba	M	3.8	1.00	0.150
45	DH293	W 1975	Suba	F	2.2	0.30	0.348
46	DH328	W 1975	Adul	M	4.6	0.40	0.462
47	DH334	W 1975	Adul	F	2.7	0.40	0.664
48	DH335	W 1975	Juve	F	1.7	0.35	0.289
49	DH342	W 1975	Adul	M	4.8	0.01	0.390
50	DH343	S 1975	Adul	M	4.1	1.15	0.156
51	DH371	S 1975	Adul	F	2.6	0.80	0.105
52	DH372	S 1975	Adul	F	2.7	0.80	0.082
53	DH388	S 1975	Suba	M	2.1	0.20	0.590
54	DH390	S 1975	Adul	F	2.5	0.35	0.283
55	DH394	S 1975	Adul	M	4.7	0.75	0.071
56	DH404	S 1975	Suba	M	2.8	0.40	0.435
57	DH421	S 1975	Suba	M	2.9	1.00	0.152
58	DH430	S 1975	Adul	M	4.1	0.42	0.700
59	DH431	S 1975	Adul	F	2.2	0.35	0.357
60	DH487	S 1975	Adul	M	3.9	0.10	0.480
61	DH497	S 1975	Adul	F	3.6	0.75	0.179
62	DH516	S 1975	Adul	M	3.7	1.00	0.207
63	DH519	S 1975	Suba	M	2.5	0.05	0.199

[a]Season during which blood sample was taken; W=winter, S=spring.

centrations than males (Fox, 1953; Henry, 1964; Needham, 1974). For example, female crabs at maturity have four times as much fat as males. Since fats act as a depository for carotenoids, this may allow female crabs to deposit more carotenes (Needham, 1974). Among mammals, the plasma of cows contains three to five times the amount found in the plasma of bulls (Semb, Baumann and Steenbock, 1934). In humans also, values for women may be slightly higher than those for men (Henry, 1964). I expected that female raccoons would have higher carotenoid levels during the late winter and early spring breeding season (McKeever, 1958) as corpora lutea, clostrum, and milk contain large amounts of these pigments (Fox, 1953).

Weights of sampled raccoons ranged from 1.7 to 5.9 kg. (3.8-13.0 lbs.). Many animals trapped in the winter of 1975 and early spring of 1975 appeared thin and weak. Several were captured by hand and, upon necropsy, showed little or no subcutaneous fat; some had heavy intestinal helminth infestations (Harman, personal commun.). These animals exhibited symptoms of canine distemper as described by Menges, Habermann and Stains, (1955) and Johnson (1970) and probably weighed less than healthy animals. A "bumper" acorn crop in the fall of 1975 (J. T. Woods, Jr., personal commun.) may have allowed heavier raccoons in the winter of 1976, as necropsy showed thick subcutaneous fat deposits.

Although sick animals did not show consis-

TABLE 3
Results of Multiple Regression Analysis on Selected Data and First Order Interactions from Selected Data from Raccoons from St. Catherines Island, Georgia

Variable	R^2 Value
Adult age class	0.05236
Subadult age class	0.00085
Juvenile age class	0
Sex	0.00070
Weight	0.02573
Season	0.02768
Distance from marsh	0.04072
Season-adult	0.02274
Season-subadult	0
Season-juvenile	NA
Season-sex	0.00514
Season-weight	0.00069
Season-distance	0.01031
Adult-sex	0.01065
Adult-weight	0.00893
Adult-distance	0.00029
Subadult-sex	0
Subadult-weight	0.00017
Subadult-distance	0.03419
Juvenile-sex	NA
Juvenile-weight	0
Juvenile-distance	0
Sex-weight	0.00115
Sex-distance	0.01699
Weight-distance	0.00095
Total	0.26024

tently low carotenoid levels, analysis of carotenoid content in raccoons may represent a reliable method for assessing nutritional condition. For example, carotenoid level in humans is correlated closely with dietary intake and provides an index of fat digestion, as well as fat absorption (Levinson and MacFate, 1969; Searcy, 1969). Carotene-poor fodder in cows causes a great decrease in milk carotenes, particularly in butterfat which becomes colorless (Lundberg, 1931). Pierce (1945) reported that sheep require 50-55 μg of carotene/kg. body weight/day to satisfy Vitamin A requirements and avoid avitaminosis. Carotenes are precursors to Vitamin A which is an important growth factor, source of visual pigments, and essential for normal structure and behavior of epithelial tissue (Searcy, 1969).

Raccoons that remained at the laboratory and were denied food and water showed a decline in carotenoid content (nos. 2, 3; 20, 21). Sample numbers 22 and 23, taken after the animal had been given water, cat food, and an egg, showed an increase in carotenoid content (table 2). Human daily variations in carotenoid content may be up to ± 50 percent and reflect dietary intake (Henry, 1964). However, Searcy (1969) reported that single meals have litte effect on carotene or serum Vitamin A concentration in humans. Further experimentation is warranted to determine if single feeding sessions influence serum carotene levels in raccoons.

Calculation of correlation coefficients revealed only nonsignificant relationships between carotenoid content and the variables tested. Location of capture and carotenoid content appeared to be somewhat related, indicating animals captured near marshes tended to have higher carotenoid levels. These higher levels may have been due to certain food items (plants, animal prey) available or more abundant in the marshes. However, reddish animals from marsh areas did not consistently show higher carotenoid levels than nonreddish animals from marsh areas. Other elements in the diet may affect the pelage color and full investigation of these, as well as environmental factors, is warranted.

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